

THE FORMATION OF THE CRANIAL SUBARACHNOID SPACES

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One of the interesting changes in viewpoint in anatomy in the last half century has been the development of a truer conception of the fluid-containing cavities of the body. From the belief that these spaces were largely alike (i.e., serous), realization has come that there exist at least two types of these peculiar fluid-sacs, excluding from the consideration synovial cavities and bursae. For the first of the two great classes, the term 'serous cavities' has been retained and is now more or less generally limited to such spaces as are derived from the coelom. None of these are to be regarded as being intimate portions of the lymphatic system; rather is their connection with this closed vascular network probably more of a functional than anatomical type.

Distinguished from this first group is the second type, comprising two fluid-systems, possessing characteristic organs of elaboration and specialized mechanisms for the absorption of their individual fluids. These cavities, the cerebro-spinal spaces and the aqueous chamber of the eye, are to be considered on these bases of a specialized mode of fluid-production and drainage, as apart from the serous cavities as they are now known. The two systems, that of the central nervous system with its cerebro-spinal fluid, and that of the eye with its aqueous humor, possess an extraordinary number of analogous processes, as pointed out by Henderson ('12) and amplified by Wegefardth and Weed ('14).

Following an investigation of the physiological anatomy of the cerebro-spinal (particularly the meningeal) spaces in the ordinary laboratory mammals (Weed, '14), attention was directed to the problems of these perimedullary cavities in the embryo.

The results of a portion of this work were reported before the Christmas meeting of the American Association of Anatomists (Weed, '16). At that time a method of replacing the existent embryonic cerebro-spinal fluid with a foreign substance was presented, permitting the substitution to be made without increasing the normal intramedullary pressure. The procedure was employed on living pig embryos, which were subsequently kept alive for varying lengths of time in a 38° incubator. Using this method and introducing an isotonic solution of potassium ferrocyanide and iron-ammonium citrate (later precipitated as Prussian blue), evidence was presented which indicated that in pig embryos the first extraventricular spread of the fluid occurred at a stage of about 14 mm. and that a total filling of the perimedullary space took place when the embryos attained a length of 26 mm.

The passage of fluid from the cerebral ventricles into the perimedullary spaces occurred only from two localized areas of ependymal differentiation in the two halves of the roof of the fourth ventricle. The superior of these areas was found to function actively during only a short period of growth; it was finally occluded by the changing relations of the developing rhombic structures. The more inferior area remains as a functional membrane during early fetal life and possibly throughout the adult existence.

The question naturally arose as to the character of the tissues into which the replaced foreign solution was carried by the production of more of the normal cerebro-spinal fluid. For the course of this foreign solution, under the experimental conditions, must be assumed to indicate exactly the course and perimedullary distribution of the true cerebro-spinal fluid. Not only is the question answered by the results of these replacements, but also the histological changes are such as to give insight into the processes concerned in the formation of the subarachnoid spaces.

Surrounding the central nervous system in the stages before the outpouring of cerebro-spinal fluid from the cerebral ventricles, is the undifferentiated perimedullary mesenchyme. This

tissue is of a loose character, forming a syncytial network of rather small mesh, but fragile. The nuclei of these cells are oval; the cytoplasm is largely devoted to the long processes which connect with adjacent cells. Adhering to these cytoplasmic strands are tiny albuminous coagula, of such amount as to be hardly noticeable; also in the meshes of the mesenchyme very small quantities of this protein-material may be identified. These albuminous coagula represent undoubtedly the albumen of the tissue-fluids in these undifferentiated stages.

This rather fine-meshed mesenchyme is the tissue into which the replaced foreign solution and the embryonic cerebro-spinal fluid passes when the elaboration of the fluid exceeds the intraventricular capacity. Shortly after the initial transit of the fluid into the extraventricular spaces (at 14 mm. in the pig), the process of differentiation of the tissue to form the final subarachnoid spaces occurs. Already the cranial blastemal condensation has become evident in the basilar regions, delimiting the loose meshes of the perimedullary mesenchyme. This undifferentiated mesenchyme, then, with the outpouring of the ventricular fluid, undergoes a metamorphosis into a far looser tissue; the process is apparently one of disruption of certain of the mesenchymal strands to form the heavier and stronger trabeculae of the subarachnoid spaces.

The first evidences of this enlargement of the mesh in the periaxial mesenchyme is met with in the region around the medulla oblongata. It becomes first noticeable in stages of from 15 to 18 mm. thus following shortly the extraventricular spread of the cerebro-spinal fluid. The first change does not concern a real disruption of the syncytial strands but resembles more the spreading apart of the cell-bodies by the introduction of more fluid into the so-called tissue-spaces. Thus in a given area of this mesenchyme under consideration, the number of nuclei will diminish as the process of differentiation proceeds.

After the length of 18 mm. is passed, at which stage a great augmentation in the extraventricular spread of the cerebro-spinal fluid occurs, the phenomenon of the disruption of the mesenchymal strands in the peribulbar tissues may be made out.

Many of these strands may be observed broken off, sacrificed to a few larger persisting trabeculae. The cells which give rise to these disrupted strands appear to recede until one of the larger surviving elements is reached, when they adhere and apparently aid in the future production of a permanent arachnoidal trabecula. But associated with this breaking down of the mesenchymal mesh and this formation of a smaller number of persisting strands, is another phenomenon of great importance. The larger meshes formed by the process appear filled with a fluid much richer in protein than were the original much smaller interstices. This is shown by the great quantity of the albuminous coagulum found, on histological examination, in all of the enlarged spaces in the tissue. The occurrence of this large amount of albuminous coagulum is apparently related directly to the distribution throughout this tissue of the embryonic cerebro-spinal fluid, for this embryonic fluid is very rich in protein-material as can be seen by the partial filling of the embryonic cerebral ventricles with the clotted albumen. In this respect, the embryonic fluid differs markedly from the adult, where the protein-content is surprisingly low.

This general plan of the formation of the subarachnoid channels attains its maximum in the transformation of the primitive mesenchymal meshes into the larger cisternae of the adult cerebro-spinal fluid. The process is best illustrated in the posterior cerebello-bulbar angle where the cisterna cerebello-medullaris is formed. The initial process of dilatation of the mesenchymal interstices is observed in embryo pigs of 18 to 20 mm.; at a stage of 23 mm., the mesenchymal strands are already broken down in part and the exposed surfaces of these are covered by an extensive albuminous coagulum. Likewise, at this length, the larger spaces are replete with similar coagula, indicating the presence of a protein-rich fluid. During the next 10 millimeters' growth, extensive changes in the future cisternae occur; the whole region is now an almost unbroken space, filled with a dense coagulum and interrupted by a few remaining mesenchymal strands. The outer portion of the cisterna is formed by a

continuous membrane, which, after somewhat further differentiation, will become the outer continuous layer of the arachnoidea.

In addition to this formation of the subarachnoid spaces in the adult through the enlargement of the embryonic mesenchymal spaces, the perimedullary mesenchyme undergoes in these same localities condensations which result ultimately in the formation of the arachnoid membrane and the trabeculae dividing up the cavum subarachnoideale. Mention has already been made of the adhesion of the cell-bodies of the disrupted mesenchymal elements to the persisting strands—the initial step apparently in the ultimate differentiation of the mesothelial cells which line these spaces. Gradually with the increasing growth of the embryo these cells seemingly become arranged in definite columns covering the persisting arachnoidal trabeculae. At the same time a differentiation of these primitive mesenchymal elements occurs, the cells ultimately being transformed into the typical cuboidal mesothelium of the subarachnoid spaces. This differentiation begins first in the basilar portions of the cranium and spreads upward, in a way similar to the course of development of the cranium and of the enlargement of the pericerebral spaces.

While such a general process as outlined accounts for the formation of the arachnoidal trabeculae and the subarachnoid spaces, it has but little bearing on the development of the outer intact membrane of the arachnoidea. This portion of the arachnoidea (which might be termed the arachnoid membrane as distinguished from the arachnoid trabeculae) first appears as a distinct line of mesenchymal condensation separating the mesenchyme into the primitive arachnoid and dura mater. This rather thin zone of cellular density in reality represents not only the outer surface of the arachnoidea but also the inner surface of the dura mater. At first these develop in close fusion but as the length of 50 mm. is attained in fetal pigs, a separation of the two membranes over the cerebral hemispheres is possible. At this stage also a mesothelial polygonal cell-pattern may be made out on the inner surface of the dura by silver nitrate reductions. With this cleavage of the two surfaces, the arachnoid

membrane rapidly differentiates, forming an intact layer over the subarachnoid spaces. The cells covering the surface membrane seem to change gradually into the cuboidal type, similar to those covering the arachnoidal trabeculae.

The general process, then, of formation of subarachnoid spaces in the cranium of the pig, concerns a dilatation of the early mesenchymal spaces, a disruption and breaking down of certain of the syncytial strands, and a survival of certain selected strands to form the permanent subarachnoid channels. In addition to this rarefaction of the mesenchyme, there is a process of condensation in this tissue, giving rise ultimately to the arachnoid membrane and reinforcing the trabeculae. Associated with the breaking apart of the syncytium of the perimedullary mesenchyme, there are invariably found large coagula of albuminous material, apparently an index of the circulation through the spaces of the embryonic cerebro-spinal fluid.

Such a view of the formation of the arachnoidea from the middle germ layer is in accord with the investigations of His ('65), of Kölliker ('79), of Farrar ('06) and others. His has described the formation in the spinal meninges of dorsal and ventral spaces, derived from the perispinal mesenchyme. Likewise, Farrar differentiates, in the spinal meninges of the chick, three laminae, the middle one of which alone becomes "the loose irregular reticulum of the pia-arachnoid." The results of Sterzi's ('01 and '02) researches on the comparative anatomy of the meninges afford an interesting comparison to the plan of development of the subarachnoid spaces outlined here. Streeter's ('16) description of the mode of formation of the perilymphatic spaces indicates that in the internal ear a similar process holds.

This report presents one of the phases of a study of the formation of the cerebro-spinal spaces. The complete communication, with many illustrations, will be published, under the title of *The Development of the Cerebro-Spinal Spaces in Pig and in Man* as Volume 5, Number 14, of the *Contributions to Embryology*, Carnegie Institution of Washington, Publication No. 225.

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BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

DIRECTORY OF FOREIGN STUDENTS IN UNITED STATES AND CANADA, 1915-1916. Published by the Committee on Friendly Relations among Foreign Students, Charles D. Hurrey, General Secretary, 124 East 28th Street, New York City. 69 pages.

STUDIES IN SURGICAL PATHOLOGICAL PHYSIOLOGY FROM THE LABORATORY OF SURGICAL RESEARCH, New York University, 1915.

Foreword. There is a growing tendency among groups of men studying different aspects of the same subject to collect and republish in one volume the results of their investigations. This practice is most helpful and it is one which will be greatly appreciated by other investigators.

The present volume of papers by associates in the Laboratory of Experimental Surgery of New York University, bearing as it does on vital subjects in scientific medicine, is a happy example of such efficient coöperative effort and one which may well be emulated by others. The contents should be familiar to every surgeon since its subject matter is representative not only of the surgery of today but of tomorrow.

WILLIAM J. MAYO.

Introduction. The contents of this volume have been arranged in accordance with the newer viewpoint that surgery should deal first with the greater problems of function and diagnosis, therapeutic progress being obviously dependent upon these; and second, with the details of method and technic. This viewpoint is presented in some detail in the article on "Medicine and the World War," in which the authors discuss the growing importance of laboratories of surgical research, not alone for teaching purposes but for the furtherance of the new science of preventive surgery.

A group of papers detailing certain studies upon the alimentary and neural canals and vertebral column follows and it will be seen that biology and particularly its branches, physiological chemistry and heredity, are the underlying basis of this work.

The next group of papers upon the Abderhalden reaction chronicle a series of efforts to apply chemistry directly to surgical diagnosis; and, whatever the final deduction, these studies are in harmony with the biological era into which surgery has passed.

Following these are the details of a series of studies of the ureteral neuro-musculature, of the function of the ureterovesical valve and of an operative procedure logically based upon these functional findings. Perhaps better than any other, this series illustrates the present tendency in surgery to substitute functional for anatomical facts as a basis for new operative therapeutics; to correlate function and morphology and to place reason above technic. One might properly speak of this trend as pragmatic surgery.

The intricate subject of shock has been studied carefully and the provisional results tend to disprove some of the most widely circulated hypotheses as to its nature and origin.

Finally, technical and miscellaneous papers are grouped together.

For the information of laymen who may examine these papers, it is stated that all the experimental work herein detailed was done upon animals after complete anesthesia and that morphine has been freely used to prevent post operative pain. Physicians and surgeons, of course, understand that no operative work can be done on animals satisfactorily except under full anesthesia.

JOHN WILLIAM DRAPER, *Editor.*

THE WANDERING CELLS IN THE LOOSE CONNECTIVE TISSUE OF THE BIRD AND THEIR ORIGIN

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The problem whether the different blood cells in the embryonic and adult organism are the products of development of various differentiated anlagen, or whether there is one common anlage, the differentiation of which is controlled by definite environmental conditions was the object of many spirited investigations. This problem is closely connected with a further question of deserving consideration, namely, as to whether the adult organism preserves a stock of undifferentiated mesenchymal cells, endowed with a faculty of polyvalent differentiation.

The purpose of this note is to summarize morphologic and experimental data recently acquired, thought to aid in the elucidation of problems announced, concerning the wandering cells in the loose connective tissue of the hen.

1. MORPHOLOGICAL DATA

As may be gathered from the title of this communication, the loose connective tissue contains permanently a certain amount of wandering cells. This is especially true for the embryonic connective tissue. Just as the circulating blood contains many kinds of differentiated blood cells, so the loose connective tissue of an adult hen includes, beside the fibroblasts, many kinds of amoeboid cells. Among these are included small lymphocytes, wandering histiotopic cells, granular wandering cells, Mast cells and occasionally plasma cells and true polymorphonuclear special leucocytes. All of these cells are found in the adult organism, but the majority of them appear in the embryo. They are numerous in the connective tissue of the adult hen. The free amoeboid cells are sometimes arranged in the form of character-

istic cords and groups. Thus in some regions the free amoeboid cells form complete layers which appear in sections as uninterrupted lines. They are densely arranged in some of the thin connective septa between small fat accumulations. The morphological structure of different kinds of cells encountered in the connective tissue of the adult hen were described by Dr. Solucha in the year 1908.

In 1908 I published the result of my studies on the embryogenesis of these elements in the chick embryo. Later this problem was worked out for the reptiles and the account of this work became a part of the work about the general haematopoiesis of the reptiles. The cells mentioned above as well as the tissue of other haematopoietic organs, all develop in the last instance from a common, undifferentiated mesenchymal anlage.

The mode of development of the mesenchymal cells into amoeboid cells is easily deducible. The long irregular processes so characteristic of the mesenchymal cells are withdrawn and the cell becomes free, potentially spherical, but very mobile. Sometimes innumerable short and irregular pseudopods cover the whole surface of the cell. Such a cell may remain as a wandering cell of the connective tissue, undoubtedly capable of further differentiation. In other cases it may immediately undergo further differentiation and assume the morphological structure of the large lymphocyte. This latter must be a very active cell, for its appearance is usually followed by a further differentiation, this depending on the environmental conditions.

The environmental conditions for the differentiation of an erythro-granulopoietic organ are more or less definite; a large amount of nutritive material, a region isolated from mechanical disturbance favor an intensive development of haemocyctoblasts. The localization within or outside the vessels determines the further differentiation of the haematocyctoblasts. A distension of the capillary net and a subsequent retarding of the blood current, offer again favorable conditions for multiplication and differentiation of the haematocyctoblasts.

It is much more difficult to define the specific environmental conditions, which direct the differentiation of the various cell

groups found scattered in the connective tissue. However, some indications show the existence of intimate relations between definite environmental conditions and differentiation. A striking example is presented by the differentiation of the Mast cells along the vessels of the omentum; these follow the vessels in whole ranges and envelop them by their long processes, which, as well as the whole cytoplasm, are filled up by metachromatic granules. An analogous intensive development of the Mast cells is seen under the epithelium of the intestine. Another instance of the interdependence of cell differentiation on the environmental conditions is offered by the development of the granulocytoblasts in the spleen of the reptiles and birds, this being chiefly confined to the pulpa in the normal embryos, while the small lymphocytes are principally developed in the external layers of the organ and in the splenic follicles.

The embryogenesis of the diffuse granulo-lymphatic tissue is easily established. The question of its reproduction in the adult organism is however obscure. Are the numerous granular and lymphatic cells in the connective tissue the direct offspring of analogous cells split off from the mesenchyme during embryonic life, securing their existence through their own reproduction? Or do these cells represent a renewal by cells brought by the blood current, or finally is the mode of their reproduction analogous to the one, observed in the embryonic development and may they not differentiate in the adult organism as well as in the embryo from the same source, namely, from young mesenchymal cells? A definite answer to these questions would greatly facilitate the solution of the problems stated at the beginning of this paper.

The peculiar structure of the granular cells of the connective tissue, which are not found in the circulating blood, speaks with great certitude for their local differentiation. The presence of mitosis in the non-granular amoeboid cells confirms the possibility of an independent reproduction of these cells in the connective tissue.

In studying the granulo-lymphatic cells in the connective tissue of the adult hen, I observed numerous small agglomerations of true lymphadenoid tissue. The center of such an ag-

glomeration is usually occupied by a mesenchymal syncytium, as described by Mall and Sabin in the case of the lymph glands of mammals. This syncytium must be considered as a young, undifferentiated tissue. Mall derived the connective reticulum from this tissue. Another line of differentiation is characteristic of the syncytium in the lymphatic nodes scattered in the loose connective tissue of the hen. This seems to consist in the development of free amoeboid cells from the mesenchymal syncytium cells. The syncytium of the small agglomerations of the lymphadenoid tissue consists of ramified cells, connected with their neighbors by rather short processes. The whole aspect of this syncytium strongly reminds one of such regions of the embryo body where the mesenchyme multiplies intensely and therefore becomes denser. Both in the embryo and in the adult organism the mesenchymal syncytium appears to be the source of the free amoeboid cells. Only the adult organism fails to show intense differentiation of free cells at the expense of the mesenchymal syncytium in the lymphnodes, as is so easily observed in the loose mesenchyme in the embryo. The multiplication of the granular and lymph cells in the connective tissue proceeds in the adult chiefly in a more homoplastic way, at the expense of intermediate stages of differentiation.

It is known that most of the birds and hens particularly do not possess special lymph glands and it is permissible to assume a homology between the numerous small agglomerations of lymphatic tissue and the granular and lymphatic cells, diffusely scattered in the connective tissue of birds on the one hand and the precisely localized lymph glands of the mammals on the other hand.

The conception of the haematopoietic organ, as established in recent years, requires not only the presence in it of proliferation and differentiation of various blood cells, but also the presence of ancestral mother cells. At the expense of such young cells in the lymphnodes the regeneration of various amoeboid cells takes place normally in the connective tissue of birds, and most intensely after loss of blood elements. Accordingly the loose connective tissue should be considered as a haematopoietic organ both in the adult organism and in the embryo.

The data referred to above are obtained as a result of the study of fixed material by the use of technical methods giving minute morphological details.

The existence of numerous granular and lymph cells diffusely scattered in the connective tissue of the hen, as well as of numerous localized centers of mesenchymal syncytium, must be admitted as uncontested facts. However, the whole line of development and differentiation is merely the result of interpretation, which may give a true understanding of the development, supposing no links in the series of observation to be omitted. But the omitting of certain data may give rise to the wildest speculations. One may recall only the possibility of admitting the small lymphocytes found in accumulations in the bone-marrow of birds, as being the common stem-cells for granular leucocytes and erythrocytes (Venzlaff). The validity of the interpretation may be accepted if they are confirmed by experimental tests.

2. EXPERIMENTAL DATA

Observations have shown the presence in the loose connective tissue of the adult hen of swarms of amoeboid cells. Small agglomerations of mesenchymal syncytium are also irregularly scattered about in the connective tissue. Around these agglomerations the amoeboid cells appear in greater numbers and the peripheral layers of the mesenchymal syncytium contain numerous free cells in the form of large lymphocytes, which apparently differentiate at the expense of the cells of the syncytium and partly reproduce themselves by mitosis.

The following questions, if possible, should be tested experimentally. a) Are the different amoeboid cells of the connective tissue the product of the development and differentiation of the mesenchymal nodes? If so, the mesenchymal nodes would appear to be stocks of undifferentiated cells, preserved in the adult organism from the time of the embryonic development and retaining their faculty of further differentiation. If by some means it were possible to destroy the differentiated cells without destroying the stem cells and if such destruction were followed by a new regeneration of differentiated cells, it would be easy to define the

source and the mode of regeneration in the adult organism. Such a destruction of lymphoid tissue was obtained by the use of the X-rays, which given in definite doses destroy chiefly the lymphoid elements, leaving the younger stem cells uninjured. An intense regeneration follows the loss of the lymphoid elements and chiefly proceeds at the expense of the cellular syncytium in the lymph-nodes. b) The second question to be tested experimentally is: What are the relations to other haemopoietic organs of the lymphoid elements found in the connective tissue? In other words, is the anlage for the amoeboid cells of the connective tissue and for the blood cells identical? This latter question may be answered affirmatively on the basis of experiment only in case, for example, we succeed in changing the actual line of differentiation of the loose mesenchyme into another one—if by means of certain intervention we may force the loose mesenchyme of the embryonic body which normally in certain regions gives rise only to lymphatic cells, to differentiate, let us say, into granular leucocytes. Such a metaplasia of the mesenchyme in the embryo body is obtainable, as will be shortly related.

Whether the adult organism preserves a stock of young mesenchymal cells is difficult to say at present. However, this assumption is true for the embryo and may be based not only on morphological observations but also on experiment.

In studying the factors of resistance to heteroplastic tissue-grafting, Dr. Murphy of the Rockefeller Institute made a very interesting observation. The tumor grafts give intense growth on the allantois of a chick embryo. The spleen graft of an adult chick made simultaneously with a cancer graft inhibits the cancer growth. At the same time the spleen of the embryo becomes much larger.

As already mentioned in my other papers, the spleen and other agglomerations of lymphatic tissue may be considered as localized hypertrophic centers of diffuse analogous processes, present in all parts of the loose connective tissue, which is especially true for the embryo.

If so, we should certainly expect, after grafting, the same changes throughout the whole mesenchyme, as are noticed in the

spleen. The changes in the spleen were not studied, but they obviously existed, for the organ became many times larger. In order to obtain uncomplicated results I made in my experiments single spleen grafts to 7-10 day embryos. If after grafting, a hypertrophy of amoeboid cells, or whatever changes they may be, were confined both to the spleen and to other mesenchymal haematopoietic anlagen and if these changes were identical in all regions, we could deduce herefrom that the mesenchyme of the whole embryo body and the mesenchyme of the spleen is equivalent.

The single grafts of adult spleen on the allantois of a chick embryo were also followed by enlargement of the host spleen. On the other hand, the study of the loose mesenchyme of the embryo body and of other haematopoietic anlagen showed intense and widespread changes. These changes consisted in the splitting off from the mesenchyme of haemocytoblasts in the form of the morphological unity of a large lymphocyte. It would be too long to mention all the regions of the embryo body where such an intense splitting takes place. I will merely state that no place exists in the embryo body where the mesenchyme does not show this process. Innumerable haemocytoblasts lie along the vessels in the form of thick cords, they are diffusely scattered in places where the mesenchyme is looser, the haemocytoblasts are crowded in groups in every skin papilla, strands of haemocytoblasts separate the smaller and larger muscle bundles and muscle fibers; even the mesenchyme in the centers of developing feathers shows an analogous splitting.

The splitting off of haemocytoblasts may be normally observed in the mesenchyme. The grafts may generalize, accelerate and intensify the same development through all the regions, where mesenchyme is found in the embryo body. *The extension of identical reactions to the whole loose mesenchyme and the mesenchymal haematopoietic organs amply confirm the value of the equivalence of the mesenchyme in the spleen, the thymus and in other parts of the embryo body, where an identical reaction takes place.*

The intense splitting off of the haemocytoblasts in the loose mesenchyme of the embryo body, of the spleen and of the

thymus is accompanied by differentiation of these cells, analogous in all these regions. The study of this differentiation gives decisive data *about the equivalence of the anlage of the lymphoid elements in the connective tissue and the anlages of other haematopoietic organs*. For the differentiation of the mesenchyme now consists in a development of granulocyto blasts and granular leucocytes, even between muscle fibers.

I cannot here analyze the causes which lead to this wonderful metaplasia of the mesenchyme. I would merely like to point out the value of the results obtained for the clear understanding of the rôle of the embryonic loose mesenchyme as of an undifferentiated haematopoietic anlage. Under normal conditions the mesenchyme gives rise to a certain number of amoeboid cells, the direct offsprings of which remain as a part of the wandering cells of the loose connective tissue in the adult organism. This process undergoes intense stimulation which here evidently depends upon resorption and circulation of certain enzymes, furnished by the grafted spleen cells. At the same time a differentiation takes place in the lymphoid cells, which in many places is normally absent.

Not only the diffuse mesenchyme of the body, but the spleen and the thymus become at certain periods after grafting centers of a most intensive true granulo-leukopoiesis (giving rise to special leucocytes).

What under these circumstances becomes of the specificity of the lympho- and leukoblasts? The same cells, which under ordinary conditions give lymphatic non-granular cells as products of their differentiation, now become the source of the most intensive leukopoiesis.

Thus, the embryonic mesenchyme appears to be a diffuse anlage for both lymphopoiesis and granuloleukopoiesis. The faculty of polyvalent development for the embryonic mesenchyme is proved by the study of the changes in the mesenchyme, resulting after the grafting of spleen from an adult chick on the allantois of the embryo.

Other important results are obtained by a closer study of the changes in the spleen occurring after grafting. These results be-

come an additional proof of other polyvalent differentiation possibilities of the mesenchyme. They demonstrate once more, that only environmental conditions direct the differentiation of the haematocytoblasts derived from the spleen mesenchyme, for, numerous haemocyto blasts may under definite conditions differentiate in the spleen into erythroblasts.

The spleen of the chick embryo is normally a lympho- and granuloleuko-poietic organ. In the earliest stages of development it appears in the form of a dense, scarcely vascularized mesenchymal anlage. The splitting off of lymphoid haemocyto blasts is gradual; the latter remain outside the vessels and many of them differentiate into granulocyto blasts. The developing vessels contain mostly differentiated erythrocytes. The development in a spleen after grafts proceeds very intensely. The splitting off of lymphoid haemocyto blasts is most active. At the time when the spleen anlage becomes vascularized, numerous lymphoid haemocyto blasts pass into the lumen of the vessels. The blood current in the spleen being at this time very slow, many of them remain here and differentiate inside the vessels into erythroblasts and erythrocytes.

This differentiation of the haemocyto blasts (that is, of the large lymphocytes), into erythroblasts inside the vessels with a very slow current again shows that the haemocyto blasts are polyvalent cells, of which the differentiation is directed by environmental conditions.

The results of the experiments confirm fully the deductions made with regard to the observations of the embryogenesis of the different blood cells.

The study of the changes in the embryo, obtained by grafting have shown that the loose mesenchyme of the embryo at certain stages is equivalent in all the regions of its localization. The mesenchyme is moreover polyvalent in its potencies of development. It may give typical connective tissue cells as well as all the different kinds of blood cells. The differentiation of the mesenchymal cells is directed by the force of external conditions, whether these conditions consist in the presence or absence of different food material, or in the special physico-chemical milieu

of the vessels. Their proliferation seems to depend upon specific stimuli, which may consist in enzyme-like material.

These experimental results give strong support to the monophyletic views regarding the origin of different blood cells, and seem to stand in contradiction to the polyphyletic origin of the various blood cells.

The completely different cells (the red and the different kind of white blood cells) are so completely different only because they present the final steps of various directions of differentiation. These cells, as such, are certainly not capable of interchanging, but, as the above described experiments have shown, the various lines of differentiation of their common stem cells may be interchanged.

THE MICROSCOPIC STRUCTURE OF THE LEG MUSCLE OF THE SEA-SPIDER, ANOPLDACTYLUS LENTUS

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SEVEN FIGURES

This contribution fulfills the second¹ part of a projected comparative microscopic study of voluntary muscle. The present aim is to devote attention to hitherto undescribed specimens. Leg and wing muscle of various insects has become classic for the demonstration of sarcoplasmic cross striation, and has furnished the chief basis for our conceptions of the detailed structure and the functional phases of voluntary striped muscle. Judging from the published illustrations of insect muscle, however, the leg muscle of the sea-spider offers by comparison a material of exceptional clearness; and for this reason alone it would seem to merit a detailed description, more especially since it furnishes in short longitudinal series a complete set of structural transition stages correlating with functional phases.

The first study in this series pertained to *Limulus* muscle (*Anat. Rec.*, vol. 10, no. 3, 1916; page 210¹). A point of peculiar interest in this connection concerns the fact that both the Xiphosura and the Pycnogonids (Pantopoda) are usually placed in 'incertae sedis' in the current text-books of zoology. A quite general concensus of opinion considers them more or less closely related, and ranks them both between Crustaceans and Arachnoids. *Limulus* muscle, however, is in appearance very much more like vertebrate than like insect muscle; while the

¹ The complete paper with illustrations will appear in a forthcoming volume from the Department of Marine Biology of the Carnegie Institution of Washington.

muscle of the marine arthropod, *Anoplodactylus*, is of the typical insect type.

The material of *Anoplodactylus lentus* Wilson (formerly known as *Phoxichilidium maxillare*) was collected from the piles of the U. S. Fish Commission Wharves at Woods Hole, Mass. Various fixing methods were employed. The material here to be described was preserved in Flemming's fluid and stained with the iron-hematoxylin picric-acid-fuchsin combination.

The leg muscle fibers are long cylinders embedded in groups in a finely granular, otherwise homogenous, 'connective tissue' magma, the sparsely scattered vesicular nuclei of which are very minute (figs. 1 and 2). (The extreme minuteness of the nuclei is characteristic of all of the sea-spider tissues; the chromatin is uniformly collected in one or several spheroidal granules.)

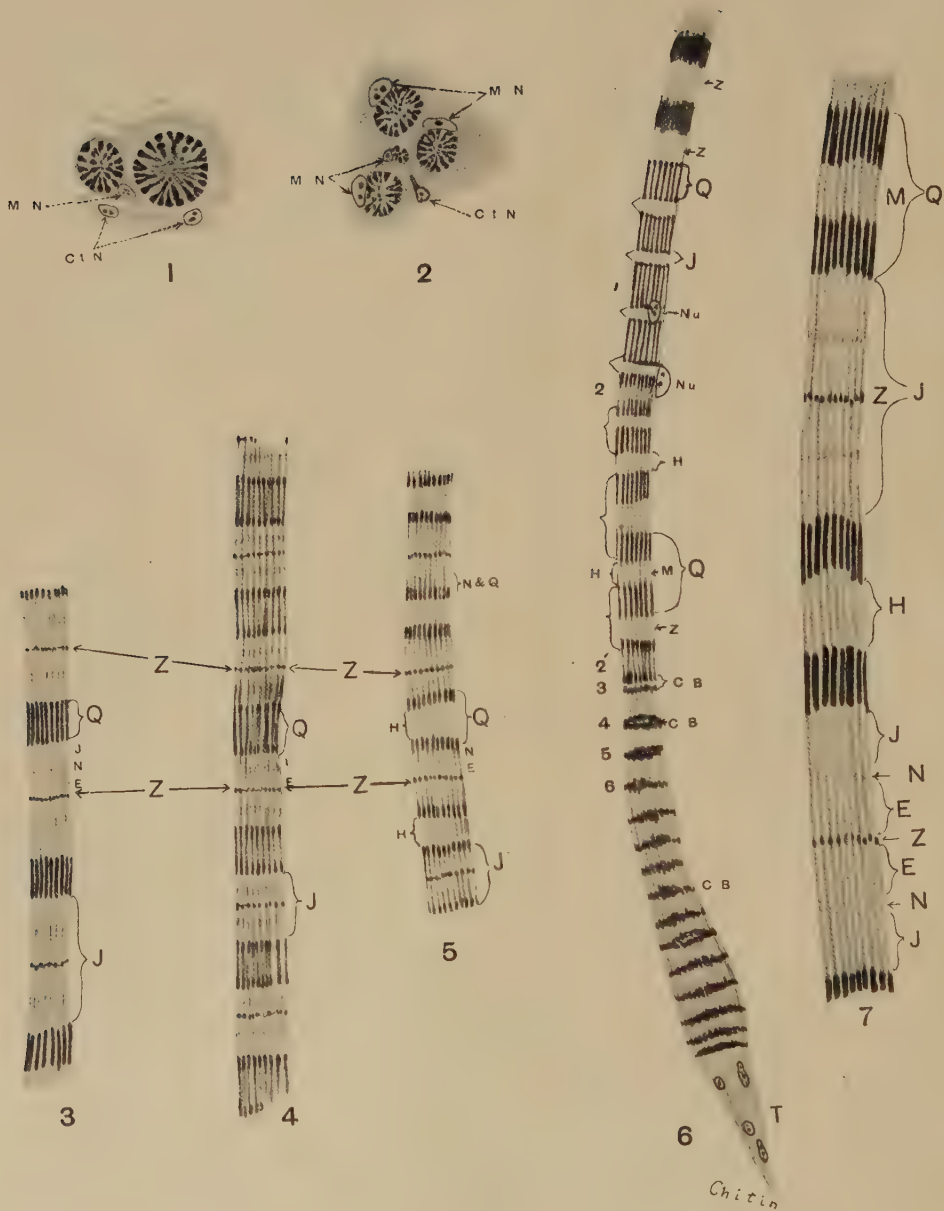
The muscle fibers in cross section have a circular or elliptical outline. They vary greatly in diameter (figs. 1 and 2); a certain amount of this variation is undoubtedly due to differences in degree of contraction and development. The fiber is invested by a meager, very delicate, layer of homogenous sarcoplasm, limited peripherally by a faint membrane, perhaps a sarcolemma. The nuclei are disposed peripherally and lie just beneath the 'sarcolemma.' Certain fibers are surrounded by a considerable space (fig. 1), a contraction artifact; when such is present the outer boundary is sharply marked by the 'sarcolemma;' the nucleus when present lies within the space. With picric-acid-fuchsin the connective tissue stains a very faint yellowish pink; the structure which I am inclined to regard as the homologue of the sarcolemma of vertebrate muscle remains unstained.

The fiber consists of a solid column of finely granular sarcoplasm throughout which are scattered the relatively coarse myofibrils. These are collected in the form of radially disposed lamellae (figs. 1 and 2) which appear to be undergoing a peripheral radial longitudinal splitting and a central tangential division. In analogy with growing *Limulus* fibers, and those of the newly-hatched rainbow trout, these appearances are most probably to be interpreted in terms of growth through myofibril increase by longitudinal fission.

In longitudinal section the prevailing fiber consists of from ten to twelve coarse myofibrils. All of the striations, with the exception of the *Z* line (telophragma), are clearly limited to the fibrils. Longitudinal sections reveal also the entire series of phases involved in the contraction process. These will be next described.

Figure 3 represents a resting, non-contracted, or relaxed fiber. Two complete sarcomeres are included. The conspicuous striping results from the *Q* discs, which consist of horizontally aligned deep-staining bacillary segments of the myofibrils. Between two successive *Q* discs appears a secondary striping in what corresponds to the *J* disc of vertebrate muscle. The *J* disc at this phase has about twice the length of the *Q* disc. The conspicuous stripe of the *J* disc is a deep-staining granular 'line' which bisects the disc, the ground membrane of Krause (*Z* stripe; telophragma). The best proof of the membrane nature of this 'stripe' which accrued from the study of the *Limulus* muscle, namely its attachment peripherally to the sarcolemma and centrally to the nuclear wall, is lacking in the case of the sea-spider muscle. What I have described above as a possible sarcolemma never shows the characteristic festooning of *Limulus* and vertebrate muscle. I am unable to determine any continuation of the *Z* stripe beyond the most peripheral myofibrils. But two observations point to its true membrane nature: 1) it has a delicate zig-zag outline, the non-granular portion passing between adjacent fibrils; 2) in cases where single peripheral fibrils are separating, the point of attachment of the separating fibril to the fiber is almost invariably at the level of the *Z* stripe. However, that the *Z* stripe is *more* than simply a membrane is indicated by the fact that its 'granules' correspond to the line of the fibrils—the myofibril apparently swells at the point of attachment to the ground membrane—and by the further fact that its character (whether granular or of sharp contour), its grade (whether fine or relatively robust) and the depth of its staining reaction vary somewhat with the phase of contraction.

Approximately midway between the *Z* line and the next succeeding *Q* disc appears a shadow of some width, the *N* line



(nebenschleibe), thus dividing this half of *J* into a central definitive *J* disc and a terminal portion of the original *J* substance, the *E* disc (endscheibe). *N* is clearly due to the presence of some

EXPLANATION OF FIGURES

Leg muscle of the Pantopod, *Anoplodactylus lentus*. The tissue was fixed in Flemming's strong solution; the sections were cut at 5 microns, and stained with iron-hematoxylin and picric-acid-fuchsin. The drawings were made by aid of a Bausch and Lomb camera lucida. Figures 1 to 6 were made with a B. & L. 1.9 mm. oil immersion objective and a number 10 ocular; figure 7 with a $\frac{1}{16}$ Leitz oil immersion lens. Figures 1 to 6 are magnified 1600 diameters; figure 7, 2000 diameters. The illustrations were reduced one-fifth in reproduction.

Fig. 1 Cross sections of two adjacent muscle fibers, enveloped by a finely granular homogeneous 'connective tissue' magma. The space about the larger fiber is a fixation artifact. *m.n.*, muscle nucleus; *ct.n.*, connective tissue nuclei. The myofibrils are arranged in radial lamellae.

Fig. 2 Cross section of a group of four adjacent muscle fibers, showing the variation in diameter and the disposition of the muscle and connective tissue nuclei.

Figs. 3, 4 and 5 Longitudinal sections of three fibers showing the structure in the relaxed condition and in two successive early stages of contraction. The fibers are so placed as to put the middle *Z* line on the same level. The arrows connecting the next series of *Z* lines above show how the sarcomeres decrease in length during contraction. A comparison of the three figures shows also how the *Q* disc lengthens and finally divides in the midline to produce the *H* disc, how each half of the original *Q* disc unites with an *N* disc in the formation of a contraction band, and how the original *J* disc disappears in the process of contraction.

Fig. 6 Longitudinal section of a complete fiber showing successive stages in contraction in longitudinal series from the relaxed condition to the tensely contracted condition. The succession of contraction phases is indicated on the left by the numerals 1 to 6. The numerals 3 to 6 designate the later steps in the formation of the contraction band, *c.b.* Both at the extreme upper end and in the mid portion (2 to 2') the fiber has suffered a stretching. In the stretched middle portion (representing a midphase of contraction), the *M* membrane is conspicuous, having apparently the same structure and staining reaction as the *Z* membrane. The brackets on the left connect the constituent elements of a future contraction band (disc). At the lower end the contracted widened fiber is connected with a chitinous midrib of the leg by tendinous tissue (*T*), the fibers of which are continuous with the myofibrils and which towards their termination show the characteristic staining reaction of collagenous fibers with acid fuchsin.

Fig. 7 Longitudinal section of a fiber showing the complete set of cross striations. Insect fibers of similar structure are generally interpreted as in the relaxed ('extended') condition. Figure 6, however, shows that a fiber of this structure represents a midphase of contraction upon which is superimposed a stretching.

substance in the myofibrils, determining a deeper staining reaction of this portion as compared with the enveloping *J* substance. It has no interfibrillar representative and can therefore not be due to the presence of sarcoplasmic granules ('*J* granules') as suggested by Heidenhain (2). Such a fiber gives not the slightest indication of the presence of an *H* disc nor of an *M* membrane (mesophragma) commonly described for the resting insect muscle fiber. The nature and significance of these stripes will be discussed below; where will appear also the proof that the fiber in question is actually in the relaxed condition.

It should perhaps be definitely stated at this point that this study proceeds upon what would seem to be a perfectly legitimate assumption, namely, that a difference in staining capacity mirrors a structural difference—if indeed perhaps only a difference in the relative amount or even concentration of physically identical material—and that a variation in structure, as revealed by staining reaction, is correlated with a phase of function. These structural differences and physical alterations are, at least in part, probably fundamentally molecular and so associated with chemical changes. The advantage that living muscle, as compared with fixed and stained material, is sometimes assumed to possess is illusory. More can apparently be learned regarding the actual morphologic changes underlying contraction with well preserved stained material than with living material. Enough can be seen from living material to give sufficient assurance that the stripes of preserved material are not fixation artifacts. Staining renders more conspicuous the actual structural and chemical alteration.

In the light of the above it would seem to be clear that the *J* disc can not be regarded as simply an optical effect, due to a difference in refractive index between *Z* and *Q* as suggested by Meigs (3) in the case of the wing muscle of the fly. It is much too wide to satisfy the requirements of such a hypothesis. Moreover, the difference in staining capacity between *Q* and *J*, and the presence of *N* render such interpretation untenable. The same argument applies in greater part also to the *H* disc as related to the *M* 'membrane' which appears in certain phases of contraction.

Figure 4 represents an early stage of contraction, figure 5 a still later intermediate stage. Figures 3, 4 and 5 are so placed as to put one *Z* line, connected by arrows, on the same level; the next *Z* line above is also connected by successive arrows. It is apparent that there occurs a gradual shortening of sarcomeres corresponding with the degree of contraction. In the phase illustrated in figure 4 the shortening is considerable. Coincident with this shortening of the sarcomeres occurs also an alteration in the *Q* disc; the other stripes remain apparently unaltered to any appreciable extent, and need not again be specially mentioned.

The change in the *Q* disc pertains to a terminal movement of *Q* substance, as indicated by the staining quality between mid-portion and termini, causing the ends to appear swollen and to become deep-staining, and the intervening portion to remain slender and to stain progressively less deeply towards the midline. It can be seen also that the *Q* disc, taken as a whole, has a slightly greater length, and the *J* disc a much shorter length, as compared with the relaxed fiber of figure 3. There is as yet no clear indication of the *H* disc and the *M* line.

An almost identical condition is illustrated also for the muscle of the antenna of the lobster by Dahlgren and Kepner (4), and a very similar condition for the muscle of the ichneumon fly larva by Prenant, Bouin et Maillard (5), for a fiber of *Dytiscus* and the wing muscle of the wasp by Schäfer (6), a muscle fiber of *Cassida equestris* by Rollet (7), and for the wing muscle of the water beetle *Hydrophilus piceus* by Ranvier (Schäfer (6)).

Figure 5 represents a later step in the same process begun in figure 4. The sarcomeres have suffered a further slight shortening; so have also the *J* discs; and *Q* has increased appreciably in length. The chief change pertains to the *Q* disc; the deeper staining constituents of this disc have moved still further apart leaving a mid-portion of considerable width unstained, the *H* disc. Moreover, the deep staining ends of the *Q* segments are no longer of spherical granular shape as in figure 4, but now have a bacillary form.

It seems clear that there has been a movement of substance from the midline of *Q* towards its terminal borders, and that the

resulting *H* disc is a phenomenon of this intermediate phase of contraction. Moreover, at a certain point in the movement the terminal portions of *Q* fuse with the *N* lines, thus obliterating the intervening portion of *J*. At a later stage in this particular phase this combined disc (*Q* + *N*) may stain deeply as a whole; this then gives the appearance frequently illustrated as the typical uncontracted ('most extended') phase (e.g., Schäfer, Quain's Anatomy, vol. 2, pt. I, p. 182, fig. 282).

As will be described in connection with figure 6 this movement of *Q* + *N* continues towards the *Z* line until *E* also disappears, and *Q* + *N* fuses with the *Z* line to form the contraction band. The latter is of course bisected by the *Z* line which has now become masked by the presence of the *Q* substance of the contraction band. At this final stage the fiber consists of a series of deep staining contraction bands separated by alternating interband unstainable segments.

In proof of the correct seriation of the three fibers just described, we may now consider a single fiber which shows the several main stages in contraction in longitudinal series.

Figure 6 illustrates a fiber contracted at its lower end; at this end it is attached to a chitinous midrib of the leg by a tendon (*T*); the opposite end, several sarcomeres beyond the last one shown, is similarly attached to the lateral chitinous wall. In passing from the attached lower end upward the contraction bands become in general less narrow and compact until at point 4 the band is clearly double. At this point also, and just above it, it may be seen that the contraction band consists of deep staining segments of the fibrils, without interfibrillar representatives except for the *Z* membrane. Conspicuous also in passing from points 4 to 5 to 6 and on towards the tendinous attachment is the gradual widening of the fiber as a whole, which is correlated with a lessening interval between successive contraction bands and a thickening also of the constituent myofibrils. Contraction clearly means, in part at least, a thickening of the fiber due to a thickening of the constituent fibrils; correlated with a movement and condensation of the *Q* substance, and a contraction in length and a rearrangement of the unstainable (*J*)

substance of the fibrils. The length of the sarcomeres at 1 is to that of the second from the end of the contracted portion as 3 to 1; the width as 1 to 2 +.

The most instructive picture with respect to contraction appears at the point 3, where the upper dark staining line of granules (similar to the condition illustrated in fig. 4) is closely apposed to a second (lower) dark staining line (similar to the condition shown in figure 5, but a somewhat later stage); and the two are about to come still closer together (as at point 4) to form a contraction band (as at point 5). The several steps in the process of contraction are shown at points 1, 2', 3, 4, 5 and 6. Points 1, 2 (and 2') and 3 + 4 show clearly conditions similar to those illustrated in the separate fibers, figures 3, 4 and 5.

Point 1 of figure 6 represents an intermediate stage between figures 3 and 4. *Q* is here characterized by a terminal row of sharply defined spherical granules, indicating the initial step in the movement of the condensing *Q* substance towards the *Z* membrane. At points 1 and 2' the migration has progressed to a stage somewhere between figures 4 and 5. At points 3, 4 and 5 are shown the final steps in the same process.

What requires further consideration in the case of this fiber is the functional interpretation of the upper end, and also of the several sarcomeres between 2 and 2'. This interpretation will be attempted after consideration of the fiber illustrated in figure 7. It should be stated here that the fibers of figures 3, 4 and 5 are closely associated in the same leg; figure 6 is from a second specimen; and figure 7 from a third. These five fibers (from three different specimens) represent all of the chief structural characteristics of the very many fibers studied in many specimens.

Figure 7 represents perhaps the rarest type of fiber. It contains all the striations, including even the *M* line. The *Z* line is conspicuous and granular; both *Q* and *J* are of considerable length; and the *M* line, while very pale and delicate, is nevertheless unmistakably present. Such structural condition is generally interpreted as that of the relaxed fiber. In the light of what we have learned from figures 3 to 6 this fiber would seem to demand interpretation in terms of a midphase of contraction. But it

corresponds strictly neither with figure 4 nor with figure 5; where, among other differences, no indication of an *M* membrane can be discerned. To anticipate what I intend to prove from figure 6, the fiber of figure 7 represents, I believe, a fiber at about the stage of contraction shown in figure 4 which has become stretched considerably under the tension of an outlying tensely contracted portion of the same fiber. An early phase of contraction could alone account, I believe, for the conspicuous presence of *H*, and a stretching for the length of the *Q* discs and the faint presence of an *M* membrane.

To return to figure 6: The lower end is clearly tensely contracted, the upper end (point 1) just passing from a relaxed condition to an early phase of contraction. Points 2 and 2' represent slightly later steps in the contraction process than point 1. In contraction the fiber clearly thickens (e.g., point 6 and below); the fiber is stouter from points 1 to 2 and from points 2' to 6 than in the intervening portion; but points 2 and 2' are at the same stage of contraction, and they represent a transition step between the phase shown at point 1 and that shown at points 3 and 4. The intervening portion then it seems must be interpreted in terms of an alteration of a structure represented at points 2 and 2'. Stretching alone offers the simplest explanation of the actual conditions. The fiber here is somewhat thinner, and the *Q* disc much longer, than in other portions of the same fiber. Also the *J* disc is here longer than in the portions just above and below. The greater length of the *Q* disc and the shortness of the *J* disc at point 1 may be explained on the basis of a fusion of *Q* with *N* in the terminal movement of *Q* substance at this phase of contraction. The two uppermost sarcomeres may be explained on the same assumption of a stretching at this point. This portion of the fiber may be conceived to have been in a relaxed condition, like that illustrated in figure 3; the stretching of the fiber due to the pull of the tensely contracted opposite end brought the adjacent fibrils so closely together that when stained the *Q* disc appears almost as a single mass, in which separate fibrils are with difficulty distinguishable.

It appears then that the fiber of figure 7 is similar to that portion of the fiber 6 between points 2 and 2'. A difference inheres

in the relatively greater length of *J*, and the granular more deeply staining character of the *Z* line. Both may possibly be explained on the basis of a greater degree of stretching in fiber figure 7, as compared with the similar portion of the fiber figure 6. Granting that the *Z* line is a true membrane, to which the myofibrils are attached, spanning the interfibrillar space (as was clearly shown in *Limulus* muscle), then in a contracting fiber—where the *Z* membrane tends to become more delicate and stain less intensely, due to its being stretched in a horizontal plane on account of the swelling of the fiber—when stretched longitudinally the *Z* membrane becomes less taut, thus increasing its bulk and consequently its discernible staining capacity.

Similarly may be explained the visibility of an *M* membrane both in fiber figure 7 and in the similar portion of fiber figure 6. Neither in fiber figure 5, where the *H* disc is wide and conspicuous, nor in the contracted portion of figure 6, can an *M* membrane be seen. In both cases a longitudinal shortening is associated with an increase in diameter; this must of necessity produce lateral tension upon a membrane which may be present spanning the diameter of the fiber. If we suppose such a membrane to be actually present, but ordinarily invisible due to the limitations of microscopic vision, then relaxation of the tension upon such a membrane, due to longitudinal stretching, might bring the membrane within the lower limits of visibility. This, I believe, may be the explanation of the appearance of an *M* line in figures 6 and 7.

It must be admitted that this explanation, however, is perhaps weakened somewhat by the delicacy and light staining reaction of also the *Z* membrane in fiber figure 6 between points 2 and 2'. Aside from this objection, however, which may be met on grounds of variability in the staining reaction of *Z* under various conditions (due to varying amounts of *Q* substance left, following a contraction band), the above explanation for the conspicuous character of *M* in fibers figures 6 and 7 would seem to offer support to Heidenhain's (2) contention that an *M* membrane (mesophragma) is invariably present in striped muscle, but below or at the limit of microscopic vision (about $0.2\ \mu$ in width). That the inferred *M* membrane, however, differs structurally from

the *Z* membrane seems clear from the fact that it does not produce a peripheral festooning of the sarcolemma in vertebrate fibers, nor is it attached centrally to processes of the nuclear wall as was shown in the case of the *Z* membrane of *Limulus* muscle, both skeletal and cardiac.

However, this latter argument for a difference in structure between the *Z* and *M* membranes may be met by the supposition—for which the appearances respecting the *M* membrane described in figures 6 and 7, and their most probable interpretation, offer a tolerably sound basis—that the *M* membrane is of such extreme delicacy or relatively lesser extensibility as to be unable to withstand the tensions demanded and met on the part of the relatively more robust and extensible *Z* membrane in the formation of festoons in the sarcolemma. Regarding the actual occurrence of an *M* membrane generally in striped muscle, as maintained by Heidenhain (2), concerning which there is much discussion and scepticism, I now incline to believe that its observation and non-observation on the part of different investigators depends upon slight individual differences in acuity of microscopic vision. My observations on the sea-spider muscle yield no evidence in support of Schneider's (8) suggestion, that the *M* stripe is a transient structure representing the initial stage in the formation of the *Q* disc following a contraction band (disc).

Before taking up the question of the interpretative value with respect to function, of the microscopic structural details above outlined, we may give attention to the manner of attachment of the muscle fiber to the chitin. We have here a clear case of muscle-tendon continuity, a condition which O. Schultze (9) maintains prevails in general even in vertebrate muscle. The evidence for the myofibril-tendofibril continuity in this muscle is twofold: 1) direct observational—the myofibril can be readily seen to continue as the tendon fibril; 2) indirect, inferential—the 'connective tissue' in which the muscle fibers are embedded is non-fibrillar, whereas the tendon connection consists of coarse fibrils, like the myofibrils, among which are many small nuclei similar to the muscle nuclei. Moreover, there is here nothing in the nature of a sufficiently coarse sarcolemma to permit of the

attachment of the robust tendon fibrils, in the manner described by Baldwin (10) for certain vertebrate muscle; and the tendon stains increasingly more deeply with fuchsin as the point of attachment to the chitin is approached.

The morphologic picture contributes the following information regarding striped muscle activity in contraction: in contraction the fiber as a whole shortens and thickens; the increase in diameter of the fiber is associated also with a thickening of the fibrils, as first maintained by Rollett (1885, (7)). There is no evidence to suggest that the interfibrillar sarcoplasm has appreciably either augmented or decreased. The thickening of the fibril in contraction involves a rearrangement mainly of the stainable *Q* substance. This *Q* substance moves terminally, causing a bisection of the original *Q* disc; this movement involves the *N* disc and proceeds as far as the *Z* membrane, where, together with a similar contribution from the next succeeding *Q* disc, is formed a contraction band. Muscular motion appears thus to be of the nature of a streaming of a portion of the myofibril protoplasm. The transfer of the *Q* substance is limited distally in contraction by the *Z* membrane, and proximally in relaxation by the *M* membrane. In contraction, halves of successive *Q* discs fuse at the *Z* line; in relaxation, halves of successive contraction bands fuse at the *M* lines. Contraction and relaxation are thus apparently functions of the internal arrangement of adjacent sarcostyles (myofibrils), coördinate action at any level being attained by the attachment of the fibrils to the *Z* and *M* membranes. The morphologic evidence then seems to support Englemann's theory (11) that muscular contraction results from an interchange of fluids (gels) between different parts of a sarcostyle (sarcomere).

The minute structure of the 'contraction bands' (discs), as illustrated in figure 6, and their relationship to the *Z* membrane is essentially identical with the simpler types of intercalated discs of vertebrate heart muscle, a circumstance which would seem to furnish strong support for my interpretation (1) of intercalated discs as 'irreversible contraction bands.' Contraction in cardiac and skeletal muscle is an essentially similar process; similar contraction bands occur in both instances. Moreover the sim-

plest types of intercalated discs are very similar to contraction bands; and both are similarly related to the *Z* membrane. Assuming an irreversibility of certain contraction bands, due perhaps to unusual strains, the simpler types of intercalated discs are readily conceived to result. From the simpler, the more complex types may be derived through the operation of the simpler mechanical stresses incident to rhythmic contraction.

The foregoing discussion assumed the identity of the deeply stainable substance and the *Q* substance. The *Q* disc is generally described as composed of anisotropic substances, the *J* disc of isotropic substance. But the segregation of these two physically different granules is by no means complete; the fibril as a whole contains anisotropic materials; these are relatively more abundant in the *Q* disc. The question then arises as to whether the streaming more deeply staining substance in contraction is an aggregation of anisotropic substance. This would be the *a priori* inference but the observations of Schäfer (with gold chlorid staining) and Englemann (with the polariscope) contradict such a conclusion. Englemann and Schäfer maintain that the anisotropic material remains segregated in the clear disc between the terminal contraction bands. However, apart from a refinement of Englemann's theory of muscular contraction, which views the movement of the intrafibrillar material in terms of an absorption of the isotropic substance by the anisotropic, this apparent contradiction between the microscopic and polariscopic evidence need offer no special difficulty.

The proximate factors in muscular contraction are: 1) nerve impulse; 2) shortening and thickening of segments of the myofibrils between successive *Z* membranes, coincident with a streaming of stainable substance from *M* on either side to *Z*. The functional unit is thus the sarcomere. The fundamental closely related phenomena are streaming and shortening.

A consideration of the various theories of muscular action—chemical, chemico-dynamical, physical, physico-chemical, electrical, etc.—or the formulation of a new theory is outside the scope of this investigation, which seeks to limit itself to a description of structural details. However, it may be stated that the rapidity of the reversible action of muscle contraction seems

an obstacle to a confident acceptance of any theory thus far proposed. If one confines himself simply to the obvious facts, namely, a movement of material from midline of sarcomere to the terminal *Z* membranes and back again to either side of the *M* membrane coincident with contraction (shortening) and relaxation (lengthening), the most extreme rapidity offers no difficulty. This 'streaming' may perhaps represent an imbibition of more fluid colloids by relatively more solid proteid gels within the sarcomeric element of the myofibril, as has been suggested; the point of absorption being marked by the advancing more deeply staining *Q* line. The shortening and the thickening of the myofibril may thus be the result of a swelling and consequent rounding up of more solid presumably oval colloidal particles. But underlying this there must of course reside a functional (energy) metabolism. This involves chemical phenomena. These are known to include oxidation of carbohydrates with an evolution of heat, and the liberation of lactic acid and carbon dioxide. The morphologic evidence would seem to indicate that attempts at interpretation of muscle function must limit attention to internal changes of the myofibrils. This evidence, namely, an apparent streaming of a tinctorially, therefore structurally and perhaps chemically, distinct substance, may be brought into accord with the now more generally prevailing theory of muscular contraction expressed in terms of alterations of surface tensions between adjacent colloidal particles in the myofibrils, by regarding the line of the 'streaming' stainable substance in its passage toward the *Z* membrane as the level of progressive chemical changes upon which the surface tension phenomena depend.

A final point concerns the significance of the complex cross striation as exhibited by the sea-spider muscle and insect wing and leg muscle in general, in terms of power and rapidity of contraction. Insect muscle is commonly regarded as highly differentiated and perfected for rapid function. A high degree of differentiation no doubt obtains, as is indicated, irrespective of the clear and complex striation, by the peripheral location of the nuclei; but that such condition is specifically closely correlated with extreme rapidity of function as exhibited by wing muscles

and certain leg muscles of insects, is clearly not the case; for the sea-spider moves its legs relatively slowly. As regards the *force* of the muscular movement it is more difficult to arrive at a conclusion since factors of relative weight and distance of movement enter here; but there appears no striking evidence that the leg of the sea-spider is capable of very powerful muscular contraction, as in the case of certain leaping insects. We appear to be on firmer ground when we speak of an arthropod type of striped muscle and a vertebrate type of striped muscle. Both are highly differentiated, with a general correspondence, but with differences in detail. Rapidity and force of contraction do not seem to be necessarily correlated with a visibly complex striation. As concerns the sea-spider and the *Limulus* muscle, the former is a typical arthropod muscle, the latter corresponds more closely to the vertebrate type. The chief points of difference concern the visible presence of the *N* disc and the *M* line and the wider and more conspicuous character of the *Q* and *J* discs in arthropod as compared with vertebrate voluntary striped muscle.

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THE CORRELATION OF CERTAIN CHEMICAL FINDINGS WITH HISTOLOGICAL STRUCTURE¹

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It may be stated that in general those substances found in tissues are the products of the cellular activity of the tissues themselves. Undoubtedly many tissue constituents have been brought there as such by the blood; but under average conditions of uniform diet the determinable integral constituents are the products of the metabolic processes of the cells and cell nuclei upon the substances brought to them by various means. The glycogen of the liver and muscles, the phosphatids of the brain and the purines of all tissues are specific enough examples to demonstrate the possibility of tissue constituents being in general the product of the specific tissue activity.

During the work on uric acid in tissues it was noted that certain tissues invariably contained more uric acid than others. This difference was so marked and so constant in occurrence whatever the kind of animal under investigation, that in order to explain these differences, recourse was had to the histological structure of the tissues in question as well as to their function.

It seems plausible therefore to divide tissues into two general classes, basing the classification upon the histological structure and the uric acid content and strengthening the comparison by a consideration of the function.

In the one group it is possible to place all those tissues whose nuclear mass is relatively great in proportion to protoplasmic mass such as liver, spleen, kidney and general glandular tissue. In the other group may be placed those tissues in whose cells the nuclear material is relatively small in proportion to protoplasm, such as muscle tissue.

In the former group we find without exception that uric acid is present in quite considerable quantity. In the latter group the uric acid content is uniformly low in amount.

Histological evidence points to the fact that glandular tissue activity is inherently bound up with nuclear activity. The changes in gland activity are accompanied by well marked changes in nuclear structure. The specific function therefore of these tissues is closely associated

¹ The work herein reported was carried on while the author was working in the Department of Bio-Chemistry of Harvard Medical School and represents a part of the work required for the Degree of Doctor of Philosophy.

if not entirely bound up with nuclear metabolism. With muscle tissue however we are unable to trace any direct histological connection between tissue activity and marked changes in nuclear structure. The main function of muscle tissue depends upon its protoplasmic metabolism. Increase in muscle activity has yet to be proven to yield an increase in uric acid excretion that can be traced to increased nuclear activity. Whereas both Mareš and Smetanka have conclusively proven that increase in glandular activity causes increase in nuclear activity and metabolism, with increase in uric acid excretion.

It is not assumed that the nuclei of muscle cells have no part in the production of uric acid; but it is considered that the nuclear activity of muscle cells is differentiated from that of gland cell nuclei in that in the former the protoplasmic activity is predominant while in the latter nuclear metabolism is the main factor in the functioning of the tissues.

From the results of the herein reported analyses the foregoing conclusions seem justified. The proposition is advanced that the comparative analysis of the tissues of an animal as regards their uric acid content gives some index of the relative nuclear activity of the tissues examined.

A survey of the appended tables demonstrates the points under discussion. The figures represent milligrams of uric acid in one hundred grams of tissue.

Distribution of uric acid in cat tissues

	BLOOD	MUSCLE	LIVER	KIDNEY	SPLEEN	LUNGS
	mg.	mg.	mg.	mg.	mg.	mg.
1	0.34	0.47	13.51	6.61	7.76	3.67
2	0.47	0.37	6.60	4.60	4.09	3.15
3	0.41	0.29	4.76		3.37	0.46
7	0.10	0.26	3.72		0.61	
8	0.12	0.15	4.10		1.98	
9	0.20	Trace	4.73		0.30	
10	0.29	0.79	2.83		4.76	
11	0.15	0.28	2.27		1.30	
13	0.83	1.20	2.54			

Rabbit

	MUSCLE	LIVER	KIDNEY	SPLEEN	LUNGS	TESTES
	mg.	mg.	mg.	mg.	mg.	mg.
1	0.72	4.96	1.02	26.60	2.89	1.43
2	0.60	5.69	0.80	10.13	1.38	

Hen

	BLOOD	MUSCLE		KIDNEY	SPLEEN	LIVER
		W	R			
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
1	4.51	1.14	1.83	21.06	10.5	27.05
2	3.68	0.68	0.74	40.59	11.19	26.59

Man

	MUSCLE	LIVER	SPLEEN
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
3	1.29	3.40	5.47
4	0.62	2.41	1.55
5	0.92	2.04	2.39
6	0.59	2.48	5.55

Dove

MUSCLE	KIDNEY	LIVER
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
3.30	52.33	15.74

Turtle

MUSCLE	LIVER
<i>mg.</i>	<i>mg.</i>
0.42 White	1.03

Cat

THYROID
<i>mg.</i>
5.71

Inasmuch as the hen and dove excrete their nitrogen as uric acid thus differentiating them from the Mammalia it would not be accurate for the purpose of the argument to include the blood and kidney analyses as examples. With these exceptions the differences as previously discussed are plainly apparent.

CONCLUSIONS

From a comparison of the relative uric acid content of body tissues with their histological structure both at rest and after activity it is possible to correlate the function of the tissues with their nuclear

activity and thus draw a line of demarcation between a predominately protoplasmic activity and a predominant nuclear activity.

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COLLATERAL ROOT CANALS AND MULTIPLE FORAMINA IN HUMAN TEETH

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THREE FIGURES

It is essential to the proper understanding and treatment of foci of systemic infection arising from the presence of infected pulp tissue confined within the root canals of devitalized teeth, that the anatomy of the root canals of human teeth be more thoroughly understood.

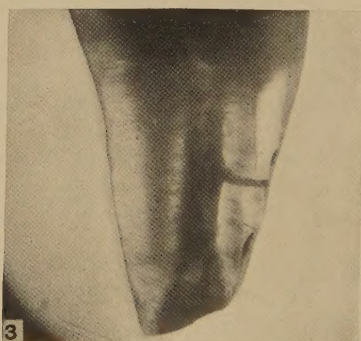
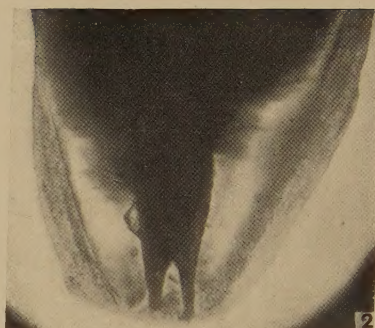
Anatomical text-books mention and illustrate the 'foramen' which transmits the afferent and efferent blood vessels and terminations of the nervous system of the pulp.

Three or more years ago Fischer discovered what appeared to be multiple foramina. Later Feiler showed, by removing cemental tissue from root ends with sand paper disks, that the multiple foramina of Fischer were canals or holes in cemental tissue produced by the action of 'antiformen' a powerful solvent of organic substance.

The surfaces of root ends so treated show that these minute canals or holes do not connect with the pulp canal, therefore are not true dental foramina.

The accompanying photomicrographs show clearly what I have for three years, in papers on root canal fillings, referred to as collateral root canals and multiple foramina.

Figures 1 and 2 are ground sections of teeth from which the pulp tissue was removed. The canals were then filled with stained rosin solution. The stained rosin solution entered the tubuli, therefore the dentine as well as the collateral canals show dark. Figure 3 is a ground section of a tooth from which pulp tissue was removed. The tooth was then placed in hematoxylin stain over night. This specimen shows a rather frequent collateral branching of the root canals in a canine tooth. I am not yet prepared to say in what per cent of teeth these canals occur.



A SHORT SPINAL CORD IN THE TOAD

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TWO FIGURES

While studying the nervous system of anura in our beginning zoology this fall, I noticed one specimen having most of the neural canal empty save for some nerves. On closer examination, it was seen that the regular ten pairs of nerves were present with their usual distribution. In each intervertebral space a pair of nerves left the neural canal. The specimen was set aside for further study.

When the central nervous system was removed entire, it was readily seen that all of the parts were present and normal except the length of the spinal cord. The letter X in figure 1 indicates the position of the sacral vertebra and the beginning of the urostyle into which the filum terminale of the spinal cord normally penetrates for a varying distance. This makes the length of the spinal cord less than one-half what it should be and was in all the others examined. During the past ten years I have had opportunity to examine more than 2000 such dissections and this is the first case of such a marked shortening.

Sections of the cord were made by using the Weigert stain and the general results are shown in figure 2. This is a cross section through the region where the seventh, eighth and ninth nerves left the stub of the cord. The following summary shows the more important modifications:

First. The neurocoele is not in the center of the section but pushed ventrally.

Second. The gray substance occupies a greater proportion of the total area in this cross section than in a spinal cord of normal length for anura.

Third. The dorsal gray commissure is greatly enlarged.

Fourth. The dorsal column is nearly double the normal size.

Fifth. There is a small funicular region.

Following Herrick's¹ classification of the somatic sensory into exteroceptive (pain, touch, heat) and proprioceptive (muscle sense); there is an interesting structural differentiation in the region illustrated by this section. The numerous exteroceptive fibers mostly end

¹ Herrick, C. J. Introduction to Neurology, 1915, p. 132.

in the dorsal column but in this region three nerves enter close together instead of each pair being separated by a vertebra. In order to be able to take care of the large amount of stimuli coming in over these three nerves, the profound changes mentioned above has taken place.

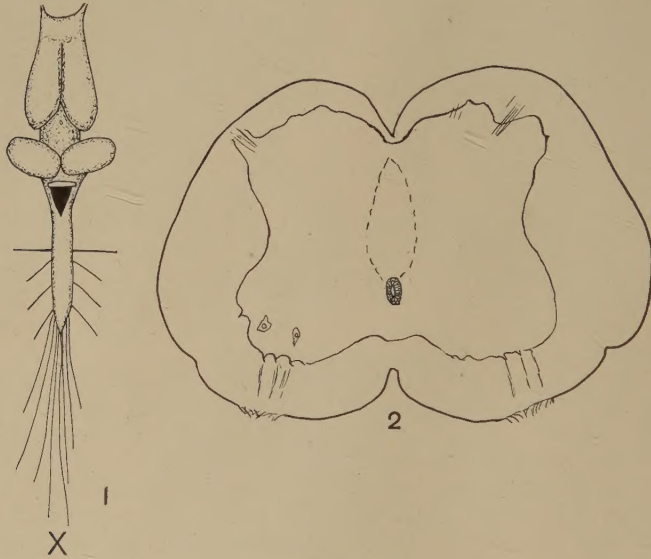


Fig. 1 Short spinal cord in a toad. The letter X indicates the position of the sacral vertebra and the beginning of the urostyle.

Fig. 2 Camera lucida drawing of cross section of spinal cord of toad through stub of cord. The area outlined with dashes is the dorsal gray commissure.